INOSITOL TRISPHOSPHATE REGULATION OF PHOTORECEPTOR MEMBRANE CURRENTS

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ABSTRACT In previous studies elevation of intracellular Ca^{2+} was shown to cause prolonged reduction of two voltage-dependent K^+ currents (I_A and $I_{Ca^{2+}-K^+}$) across the membrane of the isolated *Hermissenda* photoreceptor, the type B cell (Alkon et al., 1982*b;* Alkon and Sakakibara, 1985). Here we show that iontophoretic injection of inositol trisphosphate (IP_3), but not inositol monophosphate, also caused prolonged reduction of I_A and $I_{Ca^{2+}-K^+}$. IP_3 injection also caused reduction of a light-induced K^+ current (also $I_{Ca^{2+}-K^+}$) but did not affect the voltage-dependent Ca^{2+} current, $I_{Ca^{2+}}$, or the light-induced inward current, I_{Na^+}, of the type B cell. IP_3 injection caused similar effects on the K^+ currents of the other type of *Hermissenda* photoreceptor, the type A cell. I_{NA^+} of the type A cell, unlike that of the type B cell, was, however, markedly increased following IP_3 injection. The differences of IP_3 effects on the two types of photoreceptors may be related to differences in regulation of ionic currents by endogenous IP_3 as reflected by clear differences (before injection) in the magnitude of I_A, $I_{Ca^{2+}-K^+}$, and I_{Na^+} between the two cell types.

INTRODUCTION

Elevation of intracellular Ca^{2+}, Ca_i^{2+}, whether as a consequence of voltage-dependent flux across the cell membrane, light-induced release from intracellular stores, or iontophoretic injection, has been shown to cause prolonged reduction of K^+ currents across the soma membrane of the *Hermissenda* type B photoreceptor (Alkon et al., 1982*b;* Alkon and Sakakibara, 1985). In the most direct demonstration, Ca^{2+} was iontophoresed through one microelectrode while the isolated soma membrane potential was held at resting level (−60 mV) with voltage clamp effected by two additional microelectrodes (Alkon and Sakakibara, 1985). Two voltage-dependent outward K^+ currents, I_A and $I_{Ca^{2+}-K^+}$, measured across the soma membrane of this isolated molluscan photoreceptor, remain reduced for 2–3 min following a light paired with depolarization, many minutes after one Ca^{2+} injection (−2.0 nA, 3 min), and with larger injections for the duration of the recording period (½–1 h).

This prolonged Ca^{2+}-mediated reduction of I_A and $I_{Ca^{2+}-K^+}$ is of particular interest because these same two currents remain reduced for days following classical conditioning of intact *Hermissenda* with paired presentations of light and rotation, but not control training procedures (Alkon et al., 1982*a;* 1985). Measurement of Ca_i^{2+} by differential absorption spectrophotometry (Connor and Alkon, 1984) indicated that Ca_i^{2+} is maximally elevated within the type B photoreceptor under stimulus conditions close to those which produce the behavioral change. Other experiments have suggested that Ca^{2+}-activated phosphorylation reactions are important for producing such long-lasting reduction of I_A and $I_{Ca^{2+}-K^+}$. Reduction of I_A and $I_{Ca^{2+}-K^+}$ by a Ca^{2+} load was substantially enhanced following iontophoretic injection of two different Ca^{2+}-calmodulin-dependent kinases: phosphorylase kinase (Acosta-Urquidi et al., 1984) and Ca^{2+}-CaM type II kinase purified from brain (Sakakibara et al., 1985; 1986*a*). A similar enhancement and prolongation of Ca^{2+}-mediated reduction of I_A and $I_{Ca^{2+}-K^+}$ followed activation of C-kinase endogenous to *Hermissenda* neurons by phorbol ester or OAG (Neary et al., 1985; Alkon et al., 1986). Blockers of Ca^{2+}-CaM-dependent kinase and C-kinase such as trifluoperazine and calmidazolium produce the expected converse effects: i.e. I_A and $I_{Ca^{2+}-K^+}$ increase and the effects of enzyme injection are prevented (Sakakibara et al., 1985, 1986*a*).

Voltage-clamp conditions (e.g., a 20-s step to −5 mV paired with light) that cause prolonged Ca_i^{2+} elevation, i.e., a Ca^{2+} load, were always necessary for the effects of enzyme injection (Ca^{2+}-CaM kinase) or activation (C-kinase) to become manifest. Recent studies have shown that mobilization of Ca_i^{2+} from internal stores, such as the endoplasmic reticulum or mitochondria, is, at least in part, mediated by inositol trisphosphate, IP_3, a product of phosphatidyl inositol 4,5-bisphosphate hydrolysis (for

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review, see Berridge and Irvine, 1984). We reasoned, therefore, that prolonged reduction of I_A and $I_{Ca^{2+}-K^+}$ might follow IP_3 injection in the absence of a Ca^{2+} load, i.e., even without a depolarizing step paired with light.

The results of the present report confirm this hypothesis. I_A and, to a lesser extent, $I_{Ca^{2+}-K^+}$ across the type B soma membrane were reduced following IP_3 but not IP_1 injection in the absence of Ca^{2+} loading. $I_{Ca^{2+}-K^+}$ reduction became more apparent after a second IP_3 injection. Also, a single Ca^{2+} load caused more marked and persistent reduction of both K^+ currents following IP_3 but not IP_1 injection. Furthermore, the light-induced $I_{Ca^{2+}-K^+}$ (due to light-induced release of Ca^{2+}) was also reduced in the apparent absence of measurable change of the light-induced inward Na^+ current, I_{Na^+}. The voltage-dependent Ca^{2+} current, $I_{Ca^{2+}}$ across the type B soma membrane was also unaffected by IP_3 injection. These effects were also examined for the type of *Hermissenda* photoreceptor, the type A cell. The essential effects of IP_3 injection on I_A and $I_{Ca^{2+}-K^+}$ were similar for both types of photoreceptors, but the light-induced inward current, I_{Na^+}, of the type A (unlike that of type B) cell was markedly enhanced by IP_3 injection.

METHODS

Preparation of IP_3 and IP_1

Inositol trisphosphate and myo-inositol-1-phosphate (IP_1) were purchased from Sigma Chemical Co. (St. Louis, MO). Solutions of IP_3 (100 μM) and of IP_1 (100 μM) were prepared in a pH 9.3 carrier solution (final concentrations = 0.86 M potassium acetate, 45 mM Tris). Some experiments were conducted with 0.1–1.0 μM IP_3 in the microelectrode. The effects obtained with injection (−2.0 nA for 2 min) of low IP_3 concentration were similar to those with higher concentrations, except they often reversed within 10 min of injection.

Voltage Clamp

Photoreceptor somata were isolated by axotomy from all synaptic interactions and impulse activity as previously described (Alkon et al., 1982*a*, 1984*a*). After enzymatic digestion of an enveloping connective tissue sheath, a two-microelectrode voltage-clamp was effected by standard techniques used in preceding studies (Alkon et al., 1982*b*; Alkon and Sakakibara, 1985). Cells maintained at 20–22°C were bathed in ASW with the following composition: Na^+, 430 mM; K^+, 10 mM; Mg^{2+}, 50 mM; Ca^{2+}, 10 mM; Tris buffer (pH 7.4), 10 mM.

Separation of Ionic Currents

In darkness there are four major voltage-dependent ionic currents that flow across the type B soma membrane (Alkon et al., 1982*a, b*; 1984*a*; Alkon and Sakakibara, 1985): (*a*) I_A, an early outward voltage-dependent K^+ current, eliminated by 1–2 mM external 4-aminopyridine (4-AP); (*b*) I_K, a delayed rectifying current, not significantly activated at voltages <0 mV (absolute), and blocked by 100 mM tetraethylammonium; (*c*) $I_{Ca^{2+}}$, a sustained inward voltage-dependent current, blocked by 10 mM Cd^{2+} or Co^{2+} in the perfusing medium; and, (*d*) $I_{Ca^{2+}-K^+}$, a delayed voltage-dependent outward current eliminated by substitution of Ba^{2+} for Ca^{2+} in the perfusing medium.

In addition, illumination activates two major currents: (*a*) I_{Na^+}, a voltage-independent inward current, eliminated by removal of external Na^+ from the perfusion medium, and (*b*) $I_{Ca^{2+}-K^+}$, an outward voltage-dependent current eliminated by injection of EGTA.

In this study, I_A was measured as the peak outward current ~20 ms from the onset of the command depolarization. Because of its rapid activation, I_A is maximally activated in the almost complete absence of other voltage-dependent outward current. At 20 ms after command onset, inward calcium current offers little contamination since it is much slower to reach peak amplitude and, at −10 mV to 0 mV (absolute), is 20 to 50 times smaller than I_A. $I_{Ca^{2+}-K^+}$ was measured as the peak outward current 300–400 ms from depolarization onset. Elimination of I_A with 2 mM 4-AP or of I_K with 100 mM TEA only slightly reduces the outward current measured 300–400 msec from command onset (Alkon et al., 1984*a*). Current values included in the tables were taken both immediately before and 10 min after a Ca^{2+} load. These measurements (before and after the Ca^{2+} load) were made just prior to IP_3 injection as well as 2 min or longer after IP_3 injection. All values cited in the text are presented with standard errors of the mean. All statistical comparisons were tested with a two-tailed "paired comparison" *t* test, with the exception of Table II and type A-B comparisons made with standard two-tailed *t* test.

To assess $I_{Ca^{2+}}$ in the absence of K^+ currents, external K^+ was raised to 300 mM. This raised the equilibrium potential for K^+ flux to 0 mV (absolute) where approximately maximal activation of $I_{Ca^{2+}}$ occurs. $I_{Ca^{2+}}$ measurements were made at this K^+ reversal potential.

Iontophoretic Injection

Each injection (of control or test solution) was accomplished with two microelectrodes inserted into the type B soma. Negative current (−2.0

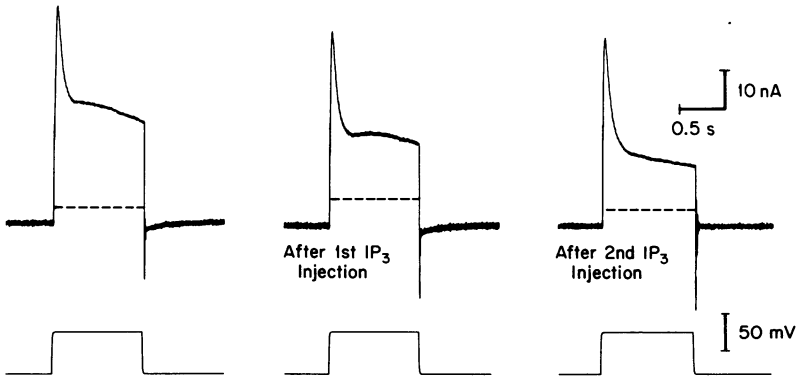

FIGURE 1 Effect of IP_3 injection on voltage-dependent outward currents across the membrane of the isolated type B photoreceptor cell soma. The currents were elicited by commands to −5 mV absolute from a holding potential of −60 mV. I_A and $I_{Ca^{2+}-K^+}$ were markedly reduced after IP_3 injection (−2.0 nA for 2 min) and further reduction occurred after a second injection. This reduction persisted for the duration of the recording. Dashed line indicates the level of the nonvoltage-dependent or "leak" current.

nA for 2 min) was passed through a microelectrode containing either IP_3 or IP_1 carrier solution. An equal but opposite current was passed through a second microelectrode filled with 3 M KCl. Thus, isopotential conditions were maintained under current clamp conditions during injection. The voltages recorded by each of the two microelectrodes were carefully measured before and after injection. Usually these voltage levels did not change and were confirmed by the potential shifts recorded at the end of the experiment on cell withdrawal. When a substantial voltage shift (≥5 mV) was recorded by the IP_3 or IP_1-filled microelectrode (following injection) and not the KCl-filled microelectrode, i.e., when the two microelectrodes recorded different voltage shifts after injection, the cell was discarded. There were, however, no changes of voltage consistently recorded by both microelectrodes as a function of injection (see Table I). Similarly, changes of holding current for voltage clamp recording (before and after injection) rarely occurred but when they exceeded 1.0 nA, the experiment was ended.

Measurements

Current values were quantitated and leak corrected as in prior studies (Alkon et al., 1982*b*, 1984*a*). All treatment conditions were repeated for type B cells from at least four different animals. The results presented are typical of those consistently obtained with such repetition.

Illumination

Light stimuli were provided by a tungsten 6V 15W bulb (Philips, Netherlands). Light was attenuated with neutral density filters to effect an intensity of $\sim 10^3$ ergs/cm^2 · s at 510 nm, at which a photosensitive peak had previously been identified for the photoreceptors (Alkon, 1976).

RESULTS

IP_3 Injection Reduces K^+ Currents

A single injection of IP_3 (−2.0 nA for 2 min) into a type B soma was followed by reduction of I_A and $I_{Ca^{2+}\text{-}K^+}$ (Figs. 1, 2; Tables I, II). Often, reduction of $I_{Ca^{2+}\text{-}K^+}$ became more clearly significant after a second IP_3 injection (Fig. 1). This reduction did not substantially reverse during the recording period (½ to 1 h). Further irreversible reduction of I_A and $I_{Ca^{2+}\text{-}K^+}$ (Tables I, II) followed a prolonged depolarizing step (to −5 mV for 25 s) paired with a 2.0 s light step (occurring 5.0 s after the depolarization onset). In the absence of IP_3, the depolarizing step paired with light (providing a Ca^{2+} load) caused only transient (2–3 min) reduction of I_A and $I_{Ca^{2+}\text{-}K^+}$. Further reduction also

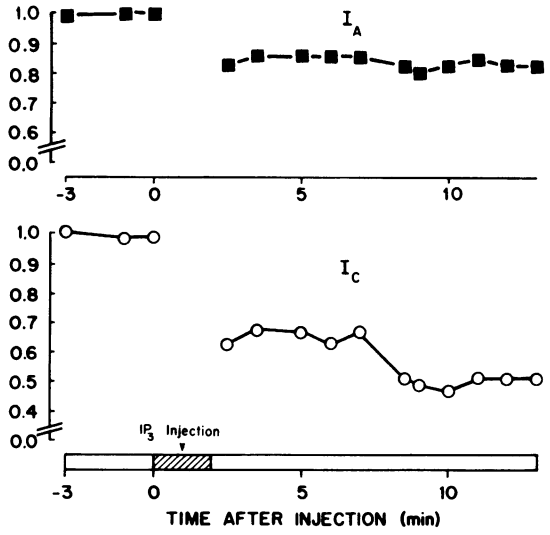

FIGURE 2 Time course of I_A and $I_{Ca^{2+}\text{-}K^+}$ reduction following IP_3 injection. I_A and $I_{Ca^{2+}\text{-}K^+}$ across type B soma membrane were measured as the peak outward currents ~20 ms (I_A) and 300–400 ms ($I_{Ca^{2+}\text{-}K^+}$) from the onset of a command depolarization to −5 mV absolute (−60 mV holding potential). I_A and $I_{Ca^{2+}\text{-}K^+}$ amplitudes before the IP_3 injection are normalized as 1.0.

TABLE I
IP_3 EFFECTS ON CURRENTS OF TYPE B CELL

Cell	Membrane potential		$I_A(-5)\|\|$			$I_{Ca^{2+}\text{-}K^+}(-5)\|\|$			$I_{Na}(-60)\|\|$		$I_{Ca^{2+}\text{-}K^+}$	
	Before	After	Before	After	Ca^{2+}-load	Before	After	Ca^{2+}-load	Before	After	Before	After
	mV	*mV*	*nA*	*nA*	*nA*	*nA*	*nA*	*nA*	*nA*	*nA*	$t_{-1/2}s$	$t_{-1/2}s$
1	−40	−40	—	—	—	9.3	8.3	5.1	7.5	7.5	9.2	10.4
2	−50	−50	19.7	18.4	17.1	12.8	10.9	9.0	6.5	6.5	7.3	7.7
3	−38	−34	21.5	20.3	—	6.8	4.8	—	—	—	—	—
4	−40	−42	23.3	19.0	—	9.9	8.5	—	—	—	9.8	10.0
5	−47	−48	20.3	17.4	13.7	16.3	14.1	11.3	2.0	3.5	4.5	5.2
6	−36	−36	26.5	22.9	22.0	13.7	9.3	7.0	—	—	—	—
7	−48	−50	16.6	16.4	11.9	17.5	14.1	12.1	—	—	4.1	5.2
8	−39	−36	22.1	18.3	12.4	11.6	9.8	5.4	—	—	5.2	7.2
9	−44	−44	20.8	17.4	13.1	10.8	8.6	5.6	4.5	4.5	7.4	9.4
10	−42	−42	16.3	13.8	11.0	10.3	8.3	5.8	6.0	8.5	—	—
11	−35	−35	17.4	11.8	6.3	7.5	5.3	2.5	7.5	7.5	—	—
Mean	−41.7	−41.5	20.5	17.6*	13.4§	11.5	9.3	7.1‡	5.7	6.3	6.8	8.3
SEM	4.73	5.68	3.01	2.97	4.29	3.21	2.83	2.95	1.93	1.77	2.09	1.8
N	11	11	10	10	8	11	11	9	6	6	7	7

*By two-tailed *t* test, $P < 0.05$.
‡$P < 0.02$.
§$P < 0.0001$.
||Peak value.

TABLE II
SUMMARY OF IP_3 AND IP_1 ON CURRENTS OF TYPE B CELL

	IP_3	vs.	IP_1	Significance value
$\Delta_1^* I_A(-5)$:	2.7 ±1.54 ($n = 10$)		−0.05 ±0.96 ($n = 6$)	$P < 0.005$
$\Delta_2^‡ I_A(-5)$:	6.5 ±2.66 ($n = 8$)		2.2 ±1.02 ($n = 3$)	$P < 0.05$
$\Delta_1^* I_{Ca^{2+}-K^+}(-5)$:	2.2 ±0.89 ($n = 11$)		0.08 ±0.79 ($n = 6$)	$P < 0.0001$
$\Delta_2^‡ I_{Ca^{2+}-K^+}(-5)$:	5.1 ±0.87 ($n = 9$)		0.67 ±0.74 ($n = 3$)	$P < 0.0001$
$\Delta I_{Na}(-50)$:	0.7 ±0.99 ($n = 6$)		1.2 ±0.74 ($n = 3$)	
$\Delta I_{Ca^{2+}-K^+}(t_{1/2})$:	1.1 ±0.66 ($n = 7$)		1.1 ±1.04 ($n = 4$)	

*Δ_1 is the difference between before the injection and after the injection.
‡Δ_2 is the difference between before the injection and after the injection, both after Ca^{2+} load.
Significance values are tested by two-tailed t test.

followed additional IP_3 injections (Fig. 1). The reduction of I_A and $I_{Ca^{2+}-K^+}$ amplitude appeared to be, at least in part, due to a reduction of the voltage-dependence of these two currents, i.e., I_A and $I_{Ca^{2+}-K^+}$ did not show as great an increase with increasingly positive voltage steps. It should be noted that when IP_3 injection was accomplished with smaller current magnitudes of briefer duration (e.g., −1.0 nA for 0.5 min) considerable reversibility of I_A and $I_{Ca^{2+}-K^+}$ reduction was usually observed. None of these effects were observed after injection of IP_1 (inositol monophosphate), a molecule with a structure related to that of IP_3, but without its biological activity in other systems. Membrane potential (−42.0 ± 5.2 vs. −41.9 ± 5.3, $N = 8$, N.S.) was not affected by IP_1 injection, nor was I_A at −5 mV (21.9 ± 3.19 vs. 20.9 ± 2.44, $N = 8$, N.S. before Ca^{2+} load, and 21.5 ± 1.5, $N = 5$, after Ca^{2+} load), $I_{Ca^{2+}-K^+}$ at −5 mV (9.4 ± 1.81 vs. 9.1 ± 2.27, $N = 8$, N.S. before Ca^{2+} load and 8.2 ± 1.83, $N = 5$, N.S., after Ca^{2+} load). IP_1 injection also had no significant effects on I_{Na^+} at −60 mV or the light-induced decrease of $I_{Ca^{2+}-K^+}$ (measured as time constant for recovery, $t_{1/2}$).

IP_3 injection also had no effect on type A cell membrane potential, but did significantly reduce I_A (15.2 ± 3.69 vs. 11.9 ± 3.04, $N = 4$, $p < 0.05$) and $I_{Ca^{2+}-K^+}$, the latter, particularly after a Ca^{2+} load (6.0 ± 0.93 vs. 3.5 ± 1.9, $N = 1.9$, $N = 4$, $P < 0.02$). The absolute magnitudes of I_A and $I_{Ca^{2+}-K^+}$ were clearly lower for the type A cells compared with values for the type B cells even before IP_3 injection. At −5 mV absolute for example, I_A was 19.9 ± 3.83 nA, $N = 17$, for type B vs. 14.5 ± 3.91, $N = 12$, for type A, $p < 0.002$. At −5 mV absolute, $I_{Ca^{2+}-K^+}$ was 11.0 ± 2.83, $N = 17$ for type B vs. 6.0 ± 1.47, $N = 12$, for type A, $p < 0.001$.

IP_3 Injection Does Not Affect Inward Ca^{2+} Current

The small (1–3 nA) sustained inward Ca^{2+} current across the photoreceptor soma membrane can be observed when there is no net flux of K^+ current (Alkon et al., 1984*a;* Alkon and Sakakibara, 1985). This can be accomplished with pharmacologic blockade of the major K^+ currents (using 4-aminopyridine, tetraethylammonium ions, and Ba^{2+} substitution for external Ca^{2+}) or by elevating external K^+ from 10 to 300 mM. With the latter, the K^+ equilibrium potential, at which there is no net K^+ current flow, is changed from −75 to 0 mV (absolute). The voltage-dependent Ca^{2+} current, $I_{Ca^{2+}}$, is maximally activated at approximately 0 mV (Alkon et al., 1984*a*) and can, therefore, be measured in 300 mM K_0^+ at 0 mV in the absence of K^+ current flux. $I_{Ca^{2+}}$ measured in this way across the type B soma membrane was unaffected by IP_3 injection (−3.8 ± 1.89 vs. −3.9 ± 1.9, $N = 7$, N.S.). $I_{Ca^{2+}}$ was also measured in 300 mM K_0^+ in the presence of blockers of the two principal outward K^+ currents: 4-aminopyridine (3 mM) to block I_A, and 10 mM Ba^{2+} substituting for Ca_0^{2+} to block $I_{Ca^{2+}-K^+}$. Here again, under these conditions $I_{Ca^{2+}}$ was not affected by IP_3 injection (−3.6 ± 1.83 vs. −3.7 ± 1.86, $N = 8$, N.S.). In 0 Na^+, 300 K^+ ASW or in 3 mM 4-AP, 10 mM Ba^{2+}-ASW, IP_3 injection had no effect on E_{K^+}, the reversal potential for K^+ flux.

IP_3 Injection Effects on I_{Na^+}

Illumination of the *Hermissenda* photoreceptors causes a voltage-independent inward Na^+ current, I_{Na^+} (Alkon, 1979; Alkon et al., 1982*a, b;* 1984*a*). This current was measured after at least 10 min dark adaptation and subsequently at 3 min intervals. The light-induced I_{Na^+} rapidly activates and inactivates (Alkon and Sakakibara, 1985). Light, in addition to causing an inward Na^+ current, elicits an outward K^+ current (Alkon et al., 1984*a;* Alkon and Sakakibara, 1985). The light-elicited K^+ current is $I_{Ca^{2+}-K^+}$ caused most likely by light-induced release of Ca^{2+} from intracellular stores (Alkon and Sakakibara, 1985). There is no light-induced inward flux of Ca^{2+}. The light-induced $I_{Ca^{2+}-K^+}$ has a time course of activation slower than that for I_{Na^+} (cf. Alkon et al., 1985). The initial peak light-induced inward current (Figs. 3, 4) at −60 mV holding potential, therefore, was taken as a measure of I_{Na^+}. IP_3 injection caused no significant change of this peak light-induced inward current across type B soma membranes (Fig. 3; Tables I, II) but did cause a clear increase (9.5 ± 0.61 vs. 19.3 ± 1.09, $N = 4$, $P < 0.001$) of the inward peak of type A cells (Fig. 4). IP_3 injection did,

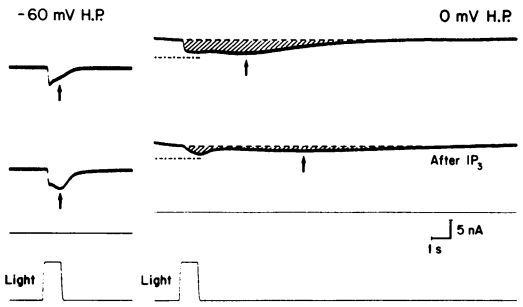

FIGURE 3 Effects of IP_3 injection on light-induced currents across type B soma membrane. One-second light steps (10^4 ergs/cm^2 · s) presented after 10-min dark adaptation at 2-min intervals, elicited (on left) inward currents (I_{Na^+}) at −60 mV. Note that the amplitude of the first phase of this inward current was unaffected by IP_3 injection (−2.0 nA, 2 min). The amplitude of the second phase (indicated by arrows on left) was increased after IP_3 injection. The same light steps also elicited prolonged "apparently" inward currents (at 0 mV) which actually were light-induced decrease of $I_{Ca^{2+}-K^+}$ (cf. Alkon and Sakakibara, 1985). The amplitude of this light-induced decrease of $I_{Ca^{2+}-K^+}$ (indicated by arrows on right) was reduced by IP_3 injection. Dot-dashed lines indicate level of "leak" or non-voltage-dependent K^+ current. Traces above those indicating light stimuli monitor voltage levels at −60 mV (on left) and 0 mV (after break, on right).

however, significantly prolong the time for relaxation (1.0 ± 0.36 vs. 1.5 ± 0.27, $N = 12$, $P < 0.001$) of the light-induced inward current of type B cells (Fig. 3). This prolonged relaxation of the light-induced inward current might reflect an IP_3 reduction of the light-induced outward current, $I_{Ca^{2+}-K^+}$. It also should be noted that the light-induced I_{Na^+} for type A cells was significantly greater than that for type B cells (9.5 ± 0.61, $N = 4$ vs. 5.7 ± 1.93, $N = 6$, $P < 0.01$) even without IP_3 injection.

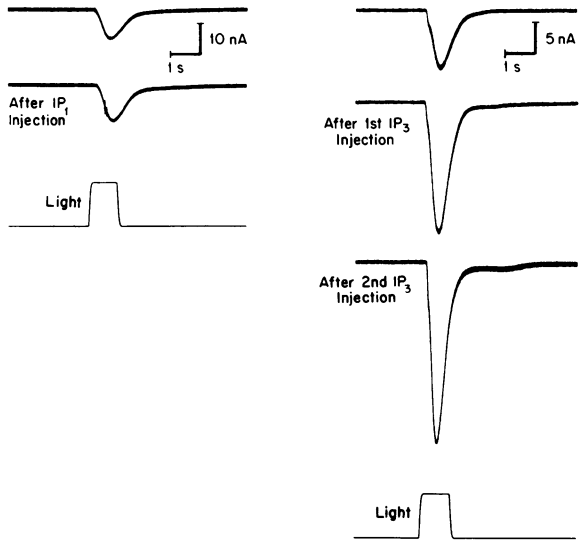

FIGURE 4 Effect of IP_3 (*right*) and IP_1 (*left*) injection on light-induced inward current, I_{Na^+}, of type A cell. One second light steps (10^4 ergs/cm^2 · s) presented after 10-min dark adaptation at 2-min intervals, elicited inward currents at a holding potential of −60 mV. Note that amplitude of I_{Na^+} increased IP_3 injection and further increase is observed after the second injection. No significant change of I_{Na^+} followed IP_1 injection.

IP_3 Injection Effects on the Light-induced Activation of $I_{Ca^{2+}-K^+}$

The light-induced $I_{Ca^{2+}-K^+}$ can be measured, in the absence of Na^+ in the external perfusion medium, as an outward current in 10 mM K_0^+ for potentials greater than or equal to 60 mV absolute or as an inward current in 300 mM K_0^+ for potentials ≤0 mV absolute. Under the latter conditions, the light-induced $I_{Ca^{2+}-K^+}$ across the type B soma membrane clearly decreased (−9.8 ± 4.71 vs. −4.4 ± 3.5, $N = 7$, $P < 0.025$) after IP_3 injection (Fig. 5).

IP_3 Injection Effects on the Light-induced Inactivation of $I_{Ca^{2+}-K^+}$

Light-induced release of Ca^{2+} from intracellular stores not only causes an initial activation of $I_{Ca^{2+}-K^+}$. It also causes a prolonged inactivation of $I_{Ca^{2+}-K^+}$ (Alkon and Sakakibara, 1985). This prolonged inactivation is manifest as an apparently inward current (Fig. 3) but is in fact a reduction of outward K^+ current ($I_{Ca^{2+}-K^+}$). IP_3 injection had no significant effect on the duration of the light-induced reduction of $I_{Ca^{2+}-K^+}$ (Tables I, II) but did decrease its magnitude (Fig. 3; 2.2 ± 0.41, $N = 8$ vs. 1.4 ± 0.73, $N = 8$, $P < 0.05$), perhaps due, in part, to the fact that steady-state $I_{Ca^{2+}-K^+}$ had already decreased following IP_3 injection.

DISCUSSION

IP_3 injection produced effects very close to those observed to persist for days during retention of the classically conditioned behavior. Type B somata isolated from classically conditioned *Hermissenda* (as compared with controls) had reduced I_A and $I_{Ca^{2+}-K^+}$ with no significant

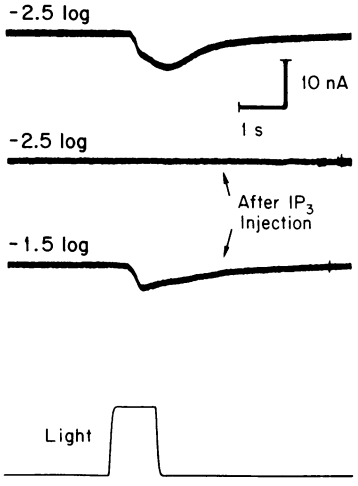

FIGURE 5 Effect of IP_3 injection of light-induced $I_{Ca^{2+}-K^+}$ across type B soma membrane. Light-induced $I_{Ca^{2+}-K^+}$ is apparent at −60 mV in 0 mM Na^+, 300 mM K_0^+-ASW. The light-induced I_{Na^+} is negligible and $I_{Ca^{2+}-K^+}$ is inward at −60 mV in this solution. IP_3 injection eliminates $I_{Ca^{2+}-K^+}$ elicited by light steps (at 10^4 ergs/cm^2 · s, indicated as −2.5 log attenuation of light source) presented at 2-min intervals. Light steps of greater intensity (indicated by −1.5 log attenuation) are still able to elicit $I_{Ca^{2+}-K^+}$ after IP_3 injection.

change of the light-induced I_{Na^+} (Alkon et al., 1982*a;* 1985). In fact, the 25–35% conditioning-induced reduction of I_A and $I_{Ca^{2+}-K^+}$ (cf. Alkon et al., 1982*a;* 1985) were quite similar to reduction magnitudes after IP_3 injection followed by a single Ca^{2+} load (cf. Table I). Injection of Ca^{2+}-CaM-II kinase after a Ca^{2+} load (Sakakibara et al., 1986) also caused persistent reduction of I_A and $I_{Ca^{2+}-K^+}$ without affecting I_{Na^+}, but it also caused a slight reduction of $I_{Ca^{2+}}$, which was unaffected by IP_3 injection. C-kinase activation had effects similar to that of IP_3, reducing (after a Ca^{2+} load) I_A and $I_{Ca^{2+}-K^+}$ without affecting I_{Na^+} or $I_{Ca^{2+}}$ (Alkon et al., 1986). The closeness of effects produced by a biochemical manipulation and classical conditioning suggests that physiologic conditions may be common to both. One such condition is the degree to which Ca_i^{2+} is elevated. It was previously found that I_A, $I_{Ca^{2+}-K^+}$ and I_{Na^+} were more sensitive to Ca_i^{2+} elevation than was $I_{Ca^{2+}}$ (Alkon et al., 1982*b,* 1984; Alkon and Sakakibara, 1985). However, the effect of Ca^{2+} elevation on I_{Na^+} appeared to involve biochemical steps distinct from those involved in Ca^{2+}'s reduction of I_A and $I_{Ca^{2+}-K^+}$ since the former but not the latter also resulted from elevation of intracellular Mg^{2+} (Alkon et al., 1982*b*).

Activation of Ca^{2+}-CaM II kinase and C-kinase endogenous to the *Hermissenda* nervous system (Neary et al., 1985) was shown to occur with Ca^{2+} levels (0.5–5 μM) not inconsistent with previous estimates of levels encountered under physiologic conditions for other cells (for review, see Rasmussen and Barrett, 1984). IP_3 injection in this study, by mobilizing Ca^{2+} from intracellular stores, may cause elevation of Ca_i^{2+} within this physiologic range sufficient to activate endogenous Ca^{2+}-CaM II kinase and perhaps, to a lesser extent, membrane-bound C-kinase as well. As suggested previously (Alkon et al., 1986) these two enzymatic pathways could act in a synergistic fashion to cause substantial and persistent reduction of I_A and $I_{Ca^{2+}-K^+}$.

Patch clamp recordings from the type B soma (Alkon et al., 1984*b,* and Sakakibara et al., 1986*b*) indicated that light-induced $I_{Ca^{2+}-K^+}$ flows across the non-rhabdomeric membrane. The light-induced I_{Na^+} flows across rhabdomeric membrane enveloped by screening pigment and exposed only near the surface of the lens and thus much less accessible to patch-clamp recording. Thus, light presumably releases Ca^{2+} from intracellular stores in the rhabdomeric part of the cell. The released Ca^{2+} would then reach the inner surface of the nonrhabdomeric membrane to activate $I_{Ca^{2+}-K^+}$, which can also be activated by Ca^{2+} elevation secondary to voltage-dependent activation of $I_{Ca^{2+}}$. In the present study, IP_3 injection enhanced I_{Na^+} of the type A cell but not the type B cell. This could be due to a difference of IP_3 effects directly on I_{Na^+} of the two cell types or indirectly to a difference of IP_3 effects on the light-induced $I_{Ca^{2+}-K^+}$ of the two cell types. If IP_3 injection reduced the light-induced $I_{Ca^{2+}-K^+}$ of the type A cell more than that of the type B cell, the type A I_{Na^+} would appear to increase since as an inward current it is opposed by the outward light-induced $I_{Ca^{2+}-K^+}$. The difference of IP_3 effects on light-induced $I_{Ca^{2+}-K^+}$ of type A and type B cells was not carefully examined in the present investigation. The light-induced $I_{Ca^{2+}-K^+}$ of the type B cell, however, was measured here in 300 mM K_0^+, 0 Na^+-ASW and found to be significantly reduced by IP_3 injection. The difference in $I_{Ca^{2+}-K^+}$ magnitude produced by IP_3 was observed in the absence of a difference in E_K. This IP_3-reduction of type B light-induced might have been expected to appear as an increased magnitude of the light-induced I_{Na^+} since the latter was measured without blocking $I_{Ca^{2+}-K^+}$. There were, however, no apparent effects of IP_3 on type B light-induced I_{Na^+} measured as the initial peak inward current, although the relaxation of the light-induced inward current was prolonged. This latter effect probably reflected IP_3 reduction of the light-induced $I_{Ca^{2+}-K^+}$. Thus, it seems likely that IP_3 injection had different effects directly on I_{Na^+} of the type A and type B photoreceptors (rather than indirectly on the light-induced $I_{Ca^{2+}-K^+}$ of the two cell types). Such a differential sensitivity of I_{Na^+} to IP_3 injection may be related to the clear differences in sensitivity to light and to conditions of light adaptation that have previously been demonstrated for type A and type B photoreceptors (Alkon and Fuortes, 1972). Greater sensitivity to IP_3 injection of the type A photoreceptor may also mean that this cell (rather than the type B photoreceptor) more closely resembles the *Limulus* ventral photoreceptors whose light-induced I_{Na^+} was also enhanced by IP_3 injection (Brown et al., 1985; Fein et al., 1985). Such an IP_3 enhancement of I_{Na^+} may be via a very different biochemical pathway than IP_3 reduction of I_A and $I_{Ca^{2+}-K^+}$. The former may be more closely related to the process of phototransduction occurring within the rhabdomeric portion of the cell while the latter (IP_3 reduction of I_A and $I_{Ca^{2+}-K^+}$) to the general integrating properties of nonrhabdomeric soma membrane.

Finally, mention should be made of the possible implications that the IP_3 effects reported here could have for understanding inactivation of $I_{Ca^{2+}-K^+}$ under physiologic conditions. One important aspect of type B $I_{Ca^{2+}-K^+}$ function is its inactivation by elevation of Ca_i^{2+} in the absence of $I_{Ca^{2+}}$ inactivation (Alkon et al., 1984*a*); Alkon and Sakakibara, 1985). Ca^{2+}-mediated inactivation of $I_{Ca^{2+}-K^+}$ without $I_{Ca^{2+}}$ inactivation is in contrast to inactivation of $I_{Ca^{2+}-K^+}$ secondary to $I_{Ca^{2+}}$ inactivation reported for other molluscan neurons (see Eckert and Lux, 1977; Eckert and Tillotson, 1978, 1981; Tillotson and Horn, 1978; Tillotson, 1979; Eckert et al., 1981). IP_3 injection, which would be expected to mobilize (and thereby elevate) Ca_i^{2+} caused I_A and $I_{Ca^{2+}-K^+}$ inactivation without affecting $I_{Ca^{2+}}$ (or I_{Na^+}). IP_3 appears, then, to work through an inactivation mechanism normally present in the type B cell, a mechanism that is dependent on Ca_i^{2+} but is independent of $I_{Ca^{2+}}$. Particular conditions of stimulation (such as a prolonged bright light or cumulative depolarization during repeated paired presentations of light and rotation) could result in particular

levels of Ca_i^{2+} for specific periods of time. These conditions of stimulation as mediated by Ca_i^{2+} elevation, and as simulated here by IP_3 injection, in turn could have expression in, and thus be encoded by, the extent and duration of I_A and $I_{Ca^{2+}-K^+}$ suppression.

Received for publication 20 May 1986.

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